Specific molecules present in cell enables to determine its properties such as function, origin, stage of differentiation. Antigenic features of these molecules are used
for their detection, especially in biomedical research and diagnostics. Specific bond between antigen and antibody can be proved by imunological methods e.g. ELISA or Western Blot. Applicability of the antibody for cell and tissue antigens needs to be tested directly by immunocytochemistry and immunohistochemistry.
Each antigen requires specific ways of fixation, processing or retrieval. These are the crucial steps for successful antigen detection. Other important steps are appropriate antibody dilution and duration and temperature of incubation.
The aim of this work was to determine the applicability of newly developed antic-Myb antibody, obtained by immunization of chick with a fragment of murine c-Myb protein. Blood serum obtained from the same animal before immunization was used as a
negative control.
Western Blot was applied to verify the specificity of our antibody. Its application for immunocytochemistry was succesfully proved on c-Myb transfected murine embryonic fibroblasts. Results of *in situ* hybridization (ISH) of *c-myb* mRNA in whole mount murine embryos compared to published data were used to define prospective c-
Myb protein positive tissues. The final step was to optimize the immunohistochemical reaction in embryonic sections.
Our ISH of *c-myb* mRNA results confirmed the expression of this gene in neuroretina, vomeronasal organ and in subperitoneal part of liver, newly also in distal phalanx of both limbs and whisker follicle.
Some of the tissues, which were published as being *c-myb* mRNA positive (according to radioactive ISH), we found to be negative (villi and crypts of intestine, proximal bronchi and trachea and subcapsular part of kidney). This can be due to the insufficient penetration of the probe to deeper situated organs of whole mount embryo.
Different methods and times of fixation, different methods of antigen retrieval and the effect of avidin-biotin amplification were compared for immunohistochemistry. Optimization of these steps enabled the detection of c-Myb protein in transverse

sections. Olfactory epithelium, vomeronasal organ, neuroretina and hematopoietic cells
of the liver proved to be c-Myb protein positive in accordance with the expression of *cmyb*
mRNA. Newly, c-Myb protein was revealed in the neural tube and in between digits. Some of tissues expressing c-myb mRNA were c-Myb protein negative when using our antibody (villi and crypts of the intestine, proximal bronchi and trachea). One
of possible explanations is that the mRNA presence not necesserily means the synthesis
of the protein.

The best results were obtained using fixation method according to Serra regardless the duration. 24 hour fixation with methacarn and short fixation (2 hours) with 4% paraformaldehyde followed by antigen retrival yielded reasonable results. Antibody dilution 1:5 000–1:10 000 and duration of incubation 60 minutes in laboratory temperature followed by 15 hours in 4 °C were set as optimal. Secondary antibody conjugated with horse-radish peroxidase was used.

Signal amplification required higher antibody dilution (1:10 000–1:15 000). However amplification did not improve the results.

We can conclude that c-Myb antibody is well applicable for Western Blot and immunocytochemistry. Its applicability for imunohistochemistry could be more problematic depending on used method of the fixation.